J. Limnol., 2014; 73(3): 562-573
DOI: 10.4081/jlimnol.2014.911

ORIGINAL ARTICLE

Environmental modulation of the plankton community composition and size-structure along the eutrophic intertidal coast of the Río de la Plata estuary, Argentina

Maximiliano D. GARCIA,[1*°] Nicolás BONEL[2]

[1]Instituto de Limnología ''Dr. Raúl A. Ringuelet", UNLP-CONICET (CCT La Plata), Boulevard 120 y 62, 1900 La Plata; [2]Laboratorio de Zoología de Invertebrados I, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. San Juan No. 670, B8000ICN Bahía Blanca, Buenos Aires, Argentina
*Corresponding author: maxidg6@yahoo.com.ar
°Present address: Laboratorio de Ecología de Zooplancton, Instituto Argentino de Oceanografía (CONICET-UNS), CCT BB, Camino La Carrindanga km 7. B8000FWB, Bahía Blanca, Buenos Aires, Argentina

ABSTRACT

*In this study we investigated the spatial distribution of the plankton community, bacterio-, phyto-, and zooplankton, in relation with environmental conditions along the intertidal coast of the Río de la Plata estuary, Argentina. Plankton was analyzed in terms of species composition, abundance, biomass (carbon content), and size-structure. We aim to evaluate the potential effects of anthropogenic impacts (*e.g.*, nutrient enrichment) and physicochemical gradients alongshore (*e.g.*, salinity, turbidity) on the composition and functioning of the plankton. We asked whether the natural structuring of the plankton by salinity and turbidity, known to be true of estuaries, is modified by eutrophication along the studied shoreline. We found that the density and biomass of bacteria and phytoplankton were strikingly enhanced by high eutrophication levels along the intertidal southwest coast of the Río de la Plata estuary. We also found that the highest zooplankton density in the most polluted area but the biomass showed a different distribution pattern. Nevertheless, when zooplankton was analyzed by means of its size fraction, we accordingly found that the microzooplankton biomass was positively associated with smaller-size phytoplankton groups and the most polluted study sites. Copepods were the major taxonomic groups that best represented the mesozooplankton biomass. We therefore expected that its distribution was modulated by the presence of its food items (*i.e.*, large cells) which, in turn, were more abundant in the middle-outer zone. In contrast, we found that the highest biomass of copepods occurred at the innermost site of the estuary and we found no significant association with other planktonic groups. Overall, this study highlights the noteworthy impacts of human activities modifying the functioning of this coastal ecosystem. The differences found in the taxonomic composition and size structure of the planktonic community assemblage between the most polluted and less polluted sites constitutes excellent baseline for considering plankton as ecological an indicator of water quality.*

Key words: Density, biomass, bacteria, phytoplankton, zooplankton, estuarine environment.

Received: November 2013. Accepted: May 2014.

INTRODUCTION

Coastal and estuarine environments are highly productive ecologic systems that are extremely interlinked with human activities (De Jonge *et al.*, 2002). Estuarine systems are therefore consummately variable and stressful aquatic biotopes in which environmental conditions can abruptly change over both space and time (González-Ortegón *et al.*, 2006). Thus, estuaries constitute dynamic ecosystems where the water circulation, anthropic impacts, and the influence of the surrounding land produce a high flux in the distribution and structure of the planktonic populations.

In estuaries, it is the variation itself in salinity (and other physical conditions such as temperature and turbidity) that can be the major source of stress to the organism (Wilson, 1994). In addition, eutrophication and organic pollution through the anthropic input from agricultural activities and domestic, industrial, and urban discharges strongly modulate plankton community biomass and composition (De Jonge *et al.*, 2002; Paerl *et al.*, 2010). Thus, in several studies plankton is considered as a useful ecological indicator to evaluate the impact of anthropogenic activities on the functioning of ecosystems in coastal environments (Beaugrand, 2005; Verlecar *et al.*, 2006). For example, anthropogenic perturbations strongly modulate the phytoplankton-community biomass and composition (Paerl *et al.*, 2010), because of its direct link and sensitivity to nutrient loading (Borja *et al*., 2012). On the other hand, in other estuaries, zooplankton response is predicting to environmental changes like salinity, water temperature, and turbidity (Marques *et al*., 2008), and some zooplanktonic species should be considered as a potential indicator of the impact of sewage (Dutto *et al*., 2012).

The Río de la Plata basin is affected by large crop areas and cattle breeding on both margins. In addition,

large urban and industrial areas surround this estuary (those being mainly the cities of Buenos Aires and Montevideo, with a total of about 13 million inhabitants) that also affect the coastal aquatic habitats and water quality (Mianzan *et al.*, 2001). Because of the extensive human population along the Río de la Plata's shores, the estuary is subjected to sewage input from large and small coastal cities, industrial waste discharges, spills of oil and other materials associated with maritime transport, and changes in the topography of the river bottom through the dredging of access channels (Framiñán and Brown, 1996). Different studies on the Río de la Plata estuary have analyzed the diversity and structure of the phytoplankton in the coastal fringe and the fluvial-mixohaline axis (Roggiero, 1988; Gómez and Bauer, 1998; Gómez *et al.*, 2002, 2004; Kogan, 2005). Moreover, environmental modulation of phytoplankton biomass and production has been extensively evaluated along a large-scale gradient between the Río de la Plata estuary and the shelf-brake off Uruguay (Calliari *et al.*, 2005, 2009). Also, Alonso *et al.* (2010) analyzed for the first time the abundance and composition of bacterioplankton along the salinity gradient occurring in the outer region of the Río de la Plata. Nevertheless, so far no ecological studies have been undertaken on the whole plankton community (*i.e.*, bacterioplankton, phytoplankton, and zooplankton) along the freshwater intertidal coast of the Río de la Plata estuary where high eutrophication and organic pollution are more influential. The main goal of this study was therefore to analyze structural changes in the coastal plankton of Río de la Plata along a natural gradient modified by nutrients and organic matter. We specifically i) analyzed spatial patterns of density and biomass of decomposers (bacteria), producers (phytoplankton), and consumers (zooplankton), ii) related those patterns to the main variables that define both the natural gradient (conductivity and turbidity) and the anthropogenic gradient (nutrient and organic matter), and iii) identified the indicators of plankton that best reflect changes in water quality. We expected that the natural structuring of the planktonic community assemblage by salinity and turbidity, known to be true of estuaries, would be significantly modified by high eutrophication levels along the intertidal coast of the Río de la Plata estuary.

METHODS

Study area

The Río de la Plata is an extensive, shallow coastal-plain estuary on the southeastern shore of South America (Mianzan *et al.*, 2001). The tidal range varies from 30 to 100 cm, tidal currents are typically below 45 cm s^{-1}, and the residence time is 46.6 days in the estuary's mixohaline zone (Guerrero *et al.*, 1997). This study was carried out along the southwest intertidal coast of the Río de Plata estuary between the coordinates 34º 27' S, 58º 30' W and 35º 27' S, 56° 45' W. Twelve intertidal sites affected by different land uses were established along 170 kilometers of the coastline (Fig. 1). The northernmost sites (S1 and

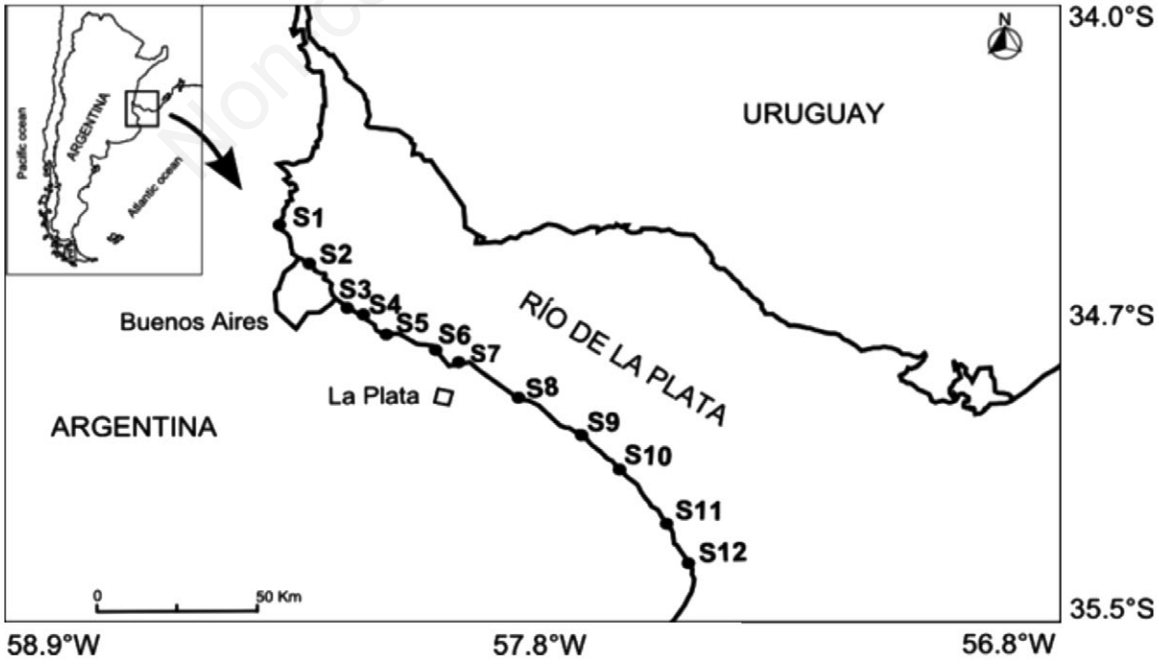

Fig. 1. Map of the Río de la Plata estuary and study sites (S).

S2) are exposed directly to the impact of human, navigational, and port activities of Buenos Aires city. Site 3 is exposed to continuous domestic- and industrial-effluent discharges into the already polluted Santo Domingo stream as well as downstream of the Matanza-Riachuelo River. Site 4 corresponds to a recreational area. Site 5 is located close to the sewage effluent of Buenos Aires city. Site 6 is exposed mainly to recreational and fishing activities. Site 7 is located on the natural reserve *Selva Marginal de Punta Lara*, though it is exposed directly to a strong discharge of domestic and industrial effluents. Site 8 is situated on the area surrounding the La Platacity sewage effluent downstream. Sites 9 through 12 are exposed to small-scale recreational and fishing activities, while the last of these sites is the closest one to the mouth of the estuary. The hydrodynamic of the study area is defined by the corridor Paraná de las Palmas River (sites 1 through 10) and the recirculation zone (sites 11 and 12; Jaime *et al.*, 2001).

Sampling procedure

Field sampling was carried out in October 2007 and May, September, and December 2008. We measured parameters *in situ*, such as water temperature, dissolved-oxygen concentration (DO), pH, conductivity, and turbidity, with a digital multiparameter Horiba sonde and estimated the salinity from the conductivity and temperature measurements. Surface water samples were collected for the analysis of dissolved nutrients: phosphate ($P\text{-}PO_4^{-3}$), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$; Mackereth *et al.*, 1978); chemical oxygen demand (COD) and biological oxygen demand (BOD_5; APHA, 1998).

To perform bacteriological analyses, we collected surface-water samples in sterile 10-mL vials and added formalin to 2% (v/v) for fixation. For the quantitative analysis of the phytoplankton, we sampled 125 mL of surface water, fixed the organisms present with 4% (v/v) formalin, and stored the samples in amber glass bottles. Finally, for the study of the zooplankton, we filtered 100 L of water through a plankton net (32-µm mesh), concentrated the sample in the bucket, and fixed the specimens with 4% (v/v) formalin. All plankton samples were collected and processed in triplicate.

Plankton abundance and biomass

We determined the abundance of the total bacteria by epifluorescence microscopy after staining the sample with 4', 6-diamino-2-phenyl-indole (DAPI) and filtration through a polycarbonate black filter of 0.2-µm pore diameter (Porter and Feig, 1980). We estimated the bacterial biomass from the biovolume and density of each bacterial form using an allometric relationship (Norland, 1993) and expressed the results in pg C utilizing the conversion factor proposed by Bratbak and Dundas (1984).

We estimated the abundance of the phytoplankton according to Lund *et al.* (1958), with an Olympus IX 51 inverted microscope at 400x in 5- to 10-mL sedimentation chambers according to the amount of suspended solids present in the sample and then identified the species with an Olympus BX 50 microscope under phase-contrast and interference optics at a magnification of 1,000x. Phytoplankton biovolume was calculated using the formula proposed by Hillebrand *et al.* (1999) and thereafter transformed into carbon content according to Menden-Deuer and Lessard (2000).

We counted zooplankton using a 1-mL Sedgwick-Rafter chamber (APHA, 1998). We estimated microzooplankton biomass based on the densities and biovolume measurements of the organisms, assuming the morphologies approximated regular geometrical shapes (Ruttner-Kolisko, 1977; Kogan, 2005). The biovolume was then converted to dry weight after Mc Cauley (1984). We estimated the dry weight of other zooplanktonic groups (cladocerans, copepods, and nematodes) using a regression formula that expressed weight as a function of length in a linear morphology (Wieser, 1960; Dumont *et al.*, 1975; Bottrell *et al.*, 1976; Riemann, 1990). For converting the data of dry weight into values of organic-carbon content, we used the factors that had been proposed for each different group: aloricate ciliates (Putt and Stoecker, 1989), tintinnids (Verity and Langdon, 1984), rotifers (Heinbokel *et al.*, 1988), copepods (Beers and Stewart, 1970), nematodes (Feller and Warwick, 1988), and cladocerans (Margalef, 1983).

Classification and data analyses

Statistical data analyses were carried out upon consideration the most representative species in abundance throughout the sampling period. Plankton was classified into different size fractions according to Margalef (1955): picoplankton (<5 µm), nanoplankton (5-50 µm), microplankton (50-500 µm), and mesoplankton (500-1000 µm). To analyze the relationship between density and biomass of major taxonomic groups of plankton and the physicochemical variables, we used independent multiple-regression analyses based on the stepwise-forward-selection method. To avoid multicollinearity effects, the salinity values were excluded from the analyses because this variable was highly correlated with conductivity (r>0.96).

We also performed two redundancy analyses (RDAs) to explore the relationship among the physicochemical variables, the study sites, and the biomass of both: the planktonic size fractions and the major taxonomic groups. These statistical analyses were performed after confirming through a preliminary detrended analysis of correspondence that the length of the gradient in units of standard de-

viation obtained was lower than 4 (Lepš and Šmilauer, 2003). The physicochemical variables selected for these analyses were conductivity, DO, turbidity, temperature, COD, BOD_5, $P\text{-}PO_4^{-3}$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, and $N\text{-}NH_4^+$ as they showed an inflation factor of variance <10 (an inflation factor of variance >10 may indicate multicolinearity between variables; Ter Braak and Verdonschot, 1995). The overall significance of the ordination and of the first two axes was tested by a Monte-Carlo permutation test ($P<0.01$) with the condition of restricted permutations.

All statistical analyses were done by means of JMP statistical software (v9.0 SAS Institute) and CANOCO software for Windows (v4.5). To meet the requirements of normality and homoscedasticity, density values were square-root transformed, whereas biomass and physicochemical data were transformed applying $\log_{10}(x+1)$.

RESULTS

Physicochemical variables

Tab. 1 summarizes the descriptive statistics of the physicochemical parameters measured at the study sites. The area was characterized by slightly alkaline water with an average value (±SD) of 8.35±0.25, though Site 3 showed the lowest mean pH (at 7.8±0.38). In addition, this site had the lowest level of dissolved oxygen (3.2±2.0 mg L^{-1}) and the highest values of BOD_5 (12.9±1.3 mg L^{-1}), COD (24.5±3.9 mg L^{-1}), $N\text{-}NH_4^+$ (1.69±0.96 mg L^{-1}), and $P\text{-}PO_4^{-3}$ (1.51±1.21 mg L^{-1}). We obtained the highest mean values for $N\text{-}NO_3^-$ at sites 4 through 7; ranging from 1.00±0.70 to 1.19±0.33 mg L^{-1} except at Site 5, where the value was somewhat higher (at 1.36±0.14 mg L^{-1}). As regards $N\text{-}NO_2^-$, Site 5 showed the highest peak with a mean value of 0.12±0.08 mg L^{-1}. The study sites 9 through 12 (those being close to the mouth of the estuary) exhibited remarkable increases in conductivity from 1,299±975 to 6,452±3,841 µS cm^{-1}, turbidity from 723±251 to 847±276 NTU, and salinity from 0.97±0.80 to 5.18±2.51 PSU.

Density and biomass of coastal plankton

A total of 332 taxa were identified, but only 57 were chosen for consideration according to the selection criteria cited in the *classification and data analyses* section. The selected taxa accounted for 89.9% of the total density (Supplementary Tab. 1).

Bacterial density revealed two main peaks at sites 3 and 7 with mean values (±SD) of 11.6±4.5x10^7 cell mL^{-1} and 7.2±1.4x10^7 cell mL^{-1}, respectively; while the bacterial biomass exhibited an equivalent pattern at the same study sites with mean values of 170.8±65.5x10^4 pg C mL^{-1} and

Tab. 1. Descriptive statistics of physicochemical variables measured in the study area.

Study sites	Water temperature (°C)	pH	Dissolved oxygen (mg L^{-1})	Biological oxygen demand (mg L^{-1})	Chemical oxygen demand (mg L^{-1})	Conductivity (µS cm^{-1})
S1	21.8±2.9	8.01±0.89	7.9±0.5	3.3±2.1	6.3±2.1	933±990
S2	21.4±3. 6	8.22±0.39	7.2±1.0	3.8±1.0	7.8±1.0	972±1,210
S3	21.5±6.6	7.87±0.38	3.2±2.0	12.9±1.3	24.5±3.9	1,059±509
S4	20.6±4.7	8.19±0.72	8.5±3.3	8.5±4.4	12.5±7.1	562±245
S5	22.7±2.0	8.33±0.50	8.0±2.4	7.3±4.6	10.8±4.2	435±100
S6	23.0±4.0	8.59±0.71	9.0±1.6	3.9±1.5	8.8±2.8	325±82
S7	23.7±4.5	8.44±0.71	9.4±1.2	6.1±6.7	11.2±7.5	294±97
S8	21.5±3.1	8.63±0.64	9.4±1.1	6.7±3.3	11.9±6.2	459±168
S9	22.6±6.3	8.63±0.56	8.9±1.1	8.4±8.3	16.3±7.5	1,300±975
S10	23.6±5.7	8.56±0.46	9.1±0.7	5.2±4.8	21.1±9.7	1,858±1,294
S11	23.1±4.2	8.26±0.34	8.4±0.4	7.9±9.4	21.6±7.3	5,180±2,368
S12	21.4±4.2	8.45±0.85	8.9±0.7	6.4±5.3	13.9±5.5	6,452±3,841
	Turbidity (NTU)	Salinity (PSU)	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Ammonium (mg L^{-1})	Phosphate (mg L^{-1})
S1	448±116	0.70±0.86	0.78±0.43	0.045±0.018	0.162±0.069	0.14±0.04
S2	366±115	0.73±1.00	0.84±0.58	0.061±0.010	0.292±0.096	0.09±0.04
S3	296±169	0.79±0.42	0.99±1.45	0.044±0.068	1.689±0.964	1.51±1.29
S4	196±58	0.42±0.20	1.00±0.70	0.084±0.079	0.787±0.879	0.49±0.51
S5	346±130	0.33±0.08	1.36±0.14	0.124±0.078	0.340±0.361	0.28±0.09
S6	201±70	0.24±0.07	1.13±0.17	0.051±0.021	0.120±0.166	0.24±0.08
S7	233±71	0.22±0.08	1.19±0.33	0.051±0.031	0.117±0.079	0.21±0.07
S8	328±37	0.34±0.13	0.49±0.45	0.005±0.004	0.005±0.005	0.16±0.08
S9	723±251	0.97±0.80	0.58±0.57	0.006±0.003	0.036±0.054	0.15±0.08
S10	753±263	1.39±1.07	0.48±0.52	0.004±0.002	0.061±0.096	0.14±0.07
S11	860±252	3.89±1.96	0.45±0.36	0.003±0.001	0.016±0.021	0.10±0.03
S12	847±276	5.18±2.51	0.35±0.25	0.012±0.009	0.069±0.106	0.11±0.05

105.6±21.5x10^4 pg C mL^{-1}, respectively (Fig. 2). According to a stepwise-multiple-regression analysis of the square-root transformed density and the log-transformed physicochemical variables, N-NO_3^- and P-PO_4^{-3}, those parameters explained 82% of the variation (density=28.7+11698.4 N-NO_3^- +17805.8 P-PO_4^{-3}; R^2=0.82, P=0.0005, n=12). Notwithstanding, zooplankton density (here ZooDens) explained 76% of variation of bacterial biomass (biomass=4.68+0.073 ZooDens; R^2=0.76, P=0.0002, n=12).

The phytoplankton density contained a single peak of 103.0±88.3x10^2 cell mL^{-1} at Site 3, while the other eleven sites exhibited density values lower than 6x10^2 cell mL^{-1}. Nevertheless, the phytoplankton biomass was elevated at sites 5 (48.4±15.4x10^3 pg C mL^{-1}) and 8 (57.9±27.6x10^3 pg C mL^{-1}) in addition to Site 3 (72.5±52.5x10^3 pg C mL^{-1}; Fig. 3). The multiple-regression analysis further indicated that P-PO_4^{-3}, Conductivity (here Cond), and zooplankton density were the best predictors of phytoplankton density (density=58.97+339.6 P-PO_4^{-3} –13.10 Cond –3.03 ZooDens; R^2 =0.98, P<0.0001, n=12). By contrast, the phytoplankton biomass was best explained by the levels of BOD_5 and pH (biomass=–4.35+2.71 BOD_5+0.77 pH; R^2=0.77, P=0.0013, n=12).

The zooplankton density increased from an average value (±SD) of 0.39±0.20x10^2 ind. L^{-1} at sites 1 and 2 to the highest densities at sites 3 and 4 (3.31±1.17 and 3.15±1.25x10^2 ind. L^{-1}, respectively). Between sites 5 and 8 the density first decreased and then remained constant at an average value of 1.54±0.50x10^2 ind. L^{-1}, whereas the sites 9 through 12 had the lowest average density (0.31±0.17x10^2 ind. L^{-1}). We recorded the highest zooplankton biomass at Site 1 (22.1±21.0x10^3 pg C mL^{-1}), while at the other sites the biomasses ranged from 2.4±1.0 to 10.4±9.3x10^3 pg C mL^{-1} (Fig. 2). Multiple-regression analysis indicated that the BOD_5 and turbidity were the

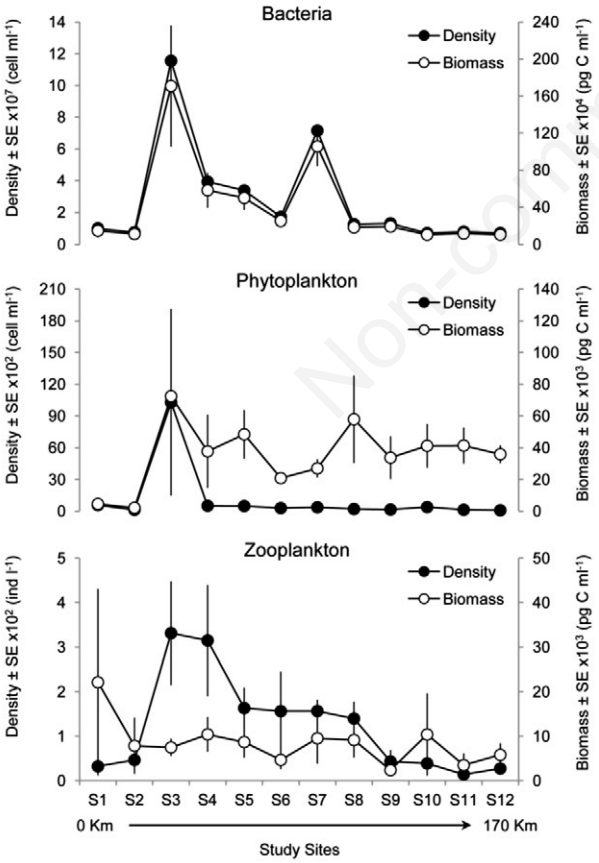

Fig. 2. Density and biomass of plankton from the intertidal coast of the Río de la Plata estuary. SE, standard error.

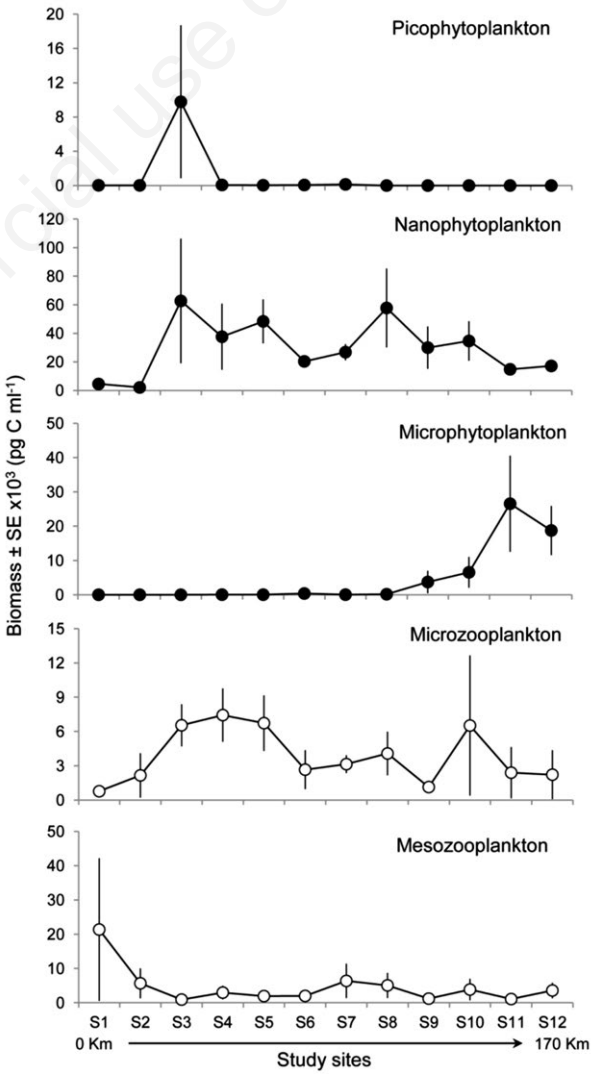

Fig. 3. Biomass of size fraction of plankton from the intertidal coast of the Río de la Plata estuary. SE, standard error.

best predictors of zooplankton density (density=43.9+15.3 BOD_5 –18.2 turbidity; R^2=0.96, P<0.0001, n=12). After entering all the variables into the multiple-regression analysis, we were unable to find a significant model to predict zooplankton biomass.

When the phytoplankton were analyzed by the size fractions of the organisms present (Fig. 3), the total biomass of the nanophytoplanktonic cells (357x10^3 pg C mL^{-1}) represented 84.3% of the total phytoplankton biomass, and the distribution among the study sites followed a pattern similar to what we had observed for the phytoplankton biomass. The biomass of the picophytoplankton represented 2.4% of the total phytoplankton biomass and exhibited a single remarkable peak at Site 3 with a mean biomass of 9.8±8.9x10^3 pg C mL^{-1} (Fig. 3). The microphytoplankton biomass accounted for 13.3% of the total phytoplankton biomass and increased from 0.2±0.1x10^3 pg C mL^{-1} at Site 8 to the highest value of 26.6±14.0x10^3 pg C mL^{-1} at Site 11 (Fig. 3). By contrast, the zooplankton biomass was composed of 45% microzooplankton and 55% mesozooplankton. The highest biomass of the microzooplankton occurred from sites 3 through 5 (6.9±2.2x10^3 pg C mL^{-1}) and at Site 10 (6.5±6.1x10^3 pg C mL^{-1}), while the lowest values were recorded at sites 1 and 9 (0.8±0.3x10^3 pg C mL^{-1} and 1.1±0.4x10^3 pg C mL^{-1}; respectively; Fig. 3). The mesozooplankton biomass was about sevenfold higher at Site 1 (21.4±20.8x10^3 pg C mL^{-1}) than the average biomass measured in the other eleven sites (3.2±2.2x10^3 pg C mL^{-1}; Fig. 3).

The major taxonomic groups of phytoplankton were: diatoms, with up to 80.5% of total phytoplankton abundance, follow by chlorophytes 14.2%, euglenophytes 3.6%, and cyanobacteria 1.7%. The diatom biomass had a variable distribution among the study sites but showed the highest peak at Site 8 (57.2±31.4x10^3 pg C mL^{-1}; Fig. 4). The chlorophytes exhibited a single peak at Site 3 with a biomass of 58.1±64.6x10^3 pg C mL^{-1} (Fig. 4). The cyanophyte profile contained two distinguishable biomass peaks at sites 1 and 3 (2.9±2.2x10^3 pg C mL^{-1} and 1.6±1.0x10^3 pg C mL^{-1}; respectively), while at the other sites the average biomass was 0.3±0.2x10^3 pg C mL^{-1} (Fig. 4). The euglenophytes also exhibited two peaks, but at sites 3 and 5, with the biomasses at those two being *ca.* twentyfold higher than the values for all the remaining study sites (Fig. 4).

Fig. 4 depicts the distribution of biomass among the major taxonomic groups of zooplankton over the different study sites. The major taxonomic groups that best represented the zooplankton biomass were the copepods at 62.7% followed by the rotifers at 22.5%, while the remaining 14.8% was explained by the biomass of the cladocerans, nematodes, aloricate ciliates, and tintinnids. The highest copepod biomass occurred at Site 1 (21.0±20.5x10^3 pg C mL^{-1}) followed by a second peak at Site 10 (9.1±9.6x10^3 pg C mL^{-1}), whereas the lowest value was recorded at Site 3 (0.6±0.6x10^3 pg C mL^{-1}). The rotifers were found to be higher at sites 3 through 5 with an average biomass of 5.4±2.5x10^3 pg C mL^{-1}, but we also registered a second peak at Site 8 of 3.1±2.5x10^3 pg C mL^{-1}. The biomass of the nematodes at Site 12 (19±1.6x10^3 pg C mL^{-1}) was *ca.* four times higher than the average biomass of this phylum in the other study sites. The cladocerans showed a single peak at Site 7 with a biomass of 1.3±1.6x10^3 pg C mL^{-1}. The aloricate-ciliate biomass also exhibited a single peak, but at Site 3 (2.6±2.2x10^3 pg C mL^{-1}). The highest biomass of the tintinnids was observed at sites 4 and 6 (1.3±1.6x10^3 pg C mL^{-1}).

Relationship between the physicochemical variables, sites, and plankton biomass

The first RDA ordered the physicochemical variables, the study sites, and the biomasses of the taxa arranged by size class along two principal components accounting for 89.3% of the total variance (Fig. 5). The analysis indicated two distinguishable groups: the first showed a significant association between different taxa belonging to small-size plankton (picophytoplankton, nanophytoplankton, and microzooplankton) that were also correlated with sites with high values of N-NH_4^+, P-PO_4^{-3}, and BOD_5; while the second related the microphytoplankton to those sites with higher conductivity and turbidity.

The first two ordination axes of the second RDA considering the biomass of the taxa classified according to the major taxonomic groups within the plankton community, accounted for *ca.* 80% of the total variance (Fig. 6). We observed a strong association between the biomass of the euglenophytes, chlorophytes, and rotifers. These taxa were also related to those sites that exhibited higher values of N-NH_4^+, P-PO_4^{-3}, and BOD_5. In contrast, the biomass of the aloricate ciliates, tintinnids, and cyanobacteria constituted a different related group that was associated to those sites mainly influenced by higher levels of N-NO_3^- and N-NO_2^-. The biomass of the copepods was grouped to those sites that had high concentrations of dissolved oxygen, while the nematodes were associated with the sites with higher levels of conductivity and turbidity.

DISCUSSION

We found that the plankton community assemblage (size fractions and major taxonomic groups) was significantly modified by high eutrophication levels along the intertidal southwest coast of the Río de la Plata estuary. We observed that high densities and biomasses of bacterioplankton, picophytoplankton (chlorophytes and cyanobacteria), and microzooplankton (rotifers, aloricate ciliates, and tintinnids) were positively related to highly eutrophic and polluted sites along the inner portion of the

Río de la Plata estuary. In that area, more than 300 point sources of pollution have been identified as the primary cause of the eutrophication through the anthropic input from domestic, industrial, and urban discharges (Kurucz *et al.*, 1998). In contrast, we found that the biomasses of the microphytoplankton (diatoms) and mesozooplankton (copepods) correlated positively with the high turbidity and conductivity levels in the estuary's outer and less polluted area.

Alonso *et al.* (2010) reported that the highest bacterial abundance ($1x10^6$ cells mL^{-1}) was found in the frontal zone of southeast region of the Río de la Plata estuary in conjunction with high concentrations of organic matter and chlorophyll a. In addition, the authors suggested that the bacterial productivity and diversity would be higher in that particular area of estuaries. Instead, we found that the highest bacterial density ($1x10^8$ cells mL^{-1}) was significantly associated to higher concentrations of nitrate and phosphate measured in that same inner part of the Río de la Plata estuary. Our estimation is far higher than the

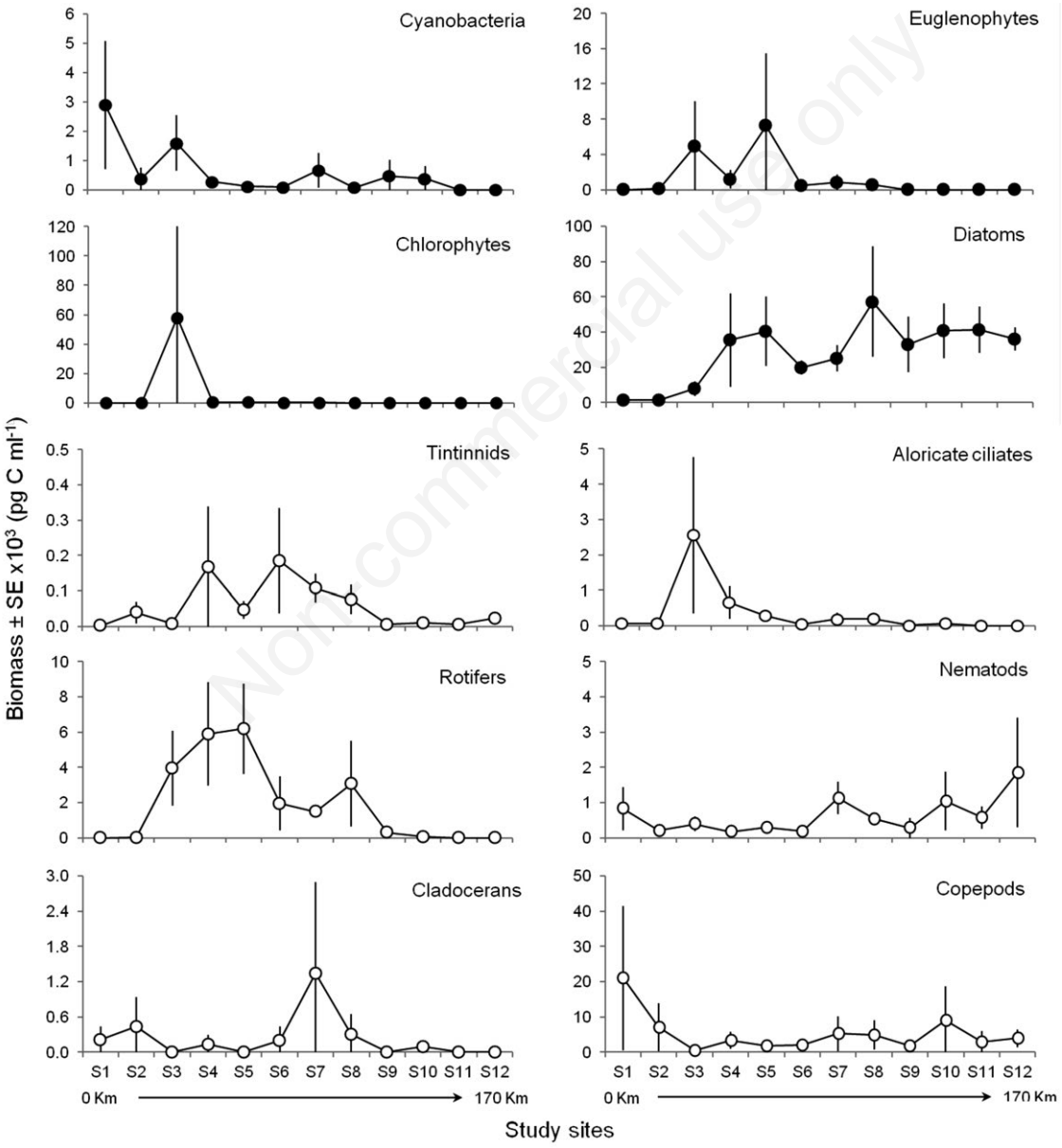

Fig. 4. Biomass of the major taxonomic groups of plankton from the intertidal coast of the Río de la Plata estuary. Black circles indicate the taxonomic groups of phytoplankton, white circles the taxonomic groups of zooplankton. SE, standard error.

bacterial abundance estimated by Alonso *et al.* (2010) and approaches to the value of $1x10^9$ cells mL^{-1} for wastewater (Hagstrøm *et al.*, 1979; Fuhrman and Azam, 1982). We assume that this atypical value estimated for the most polluted area could be related to other variables, besides that positive correlation with nitrate and phosphate, not included in this study. For instance, a higher availability of organic substrates resulting from anthropic pollution and/or originated *in situ* by other plankton groups could also significantly increase bacterial abundance. We therefore believe that this atypical value could be considered as an useful indicator of the water quality conditions in that particular area of the estuary

The average phytoplankton density (*ca.* 1,100 cell mL^{-1}) along the intertidal coast of the Río de la Plata estuary was ten-times higher than the density observed along the fluvial-mixohaline axis (Gómez *et al.*, 2004) and four-times higher than along the coastal fringe (Gómez and Bauer, 1998) in the same system. In these studies, however, the authors observed that density significantly increase in environmentally eutrophic sites located in the upper zone of the Río de la Plata. We also observed that phytoplankton density was higher at the most polluted area. Nevertheless, our estimation was twentyfold higher than the highest density observed by Gómez *et al.* (2004), three-times higher than that estimated by Gómez and Bauer (1998), and about thirtyfold higher than the average value estimated for the other eleven sites in this study. In contrast, the density was lower in the outer portion of the estuary mouth, where the salinity level was higher. The density of phytoplankton has been reported to be enhanced by the input of inorganic nutrients as consequence of anthropic impact and by a decreased abundance of phytoplankton consumers as a result of low oxygen levels (Paerl *et al.*, 2003; Kromkamp and Peene, 2005). Likewise, we found that density was positively correlated with $P\text{-}PO_4^{-3}$ levels, but negatively so with conductivity and zooplankton density. Site 3 is characterized by the highest level of eutrophication and organic pollution and, as such, dissolved oxygen level would be a limiting variable. We therefore believe that the condition of significantly low-dissolved oxygen at that site probably had negatively affected the abundance of phytoplankton consumers and, in that way, facilitated the development of high densities of phytoplankton.

The average phytoplankton biomass over the entire in-

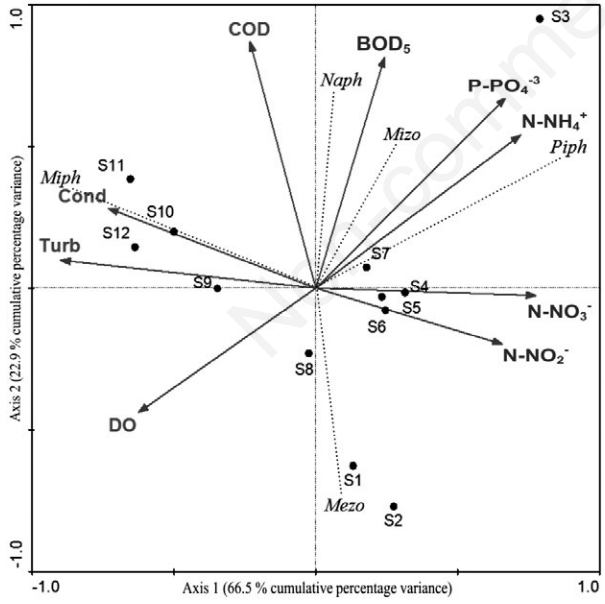

Fig. 5. Redundancy-analysis (RDA) triplot of biomass of plankton size fraction, the study sites (S), and the physicochemical variables. DO, dissolved oxygen (mg L^{-1}); BOD_5, biological oxygen demand (mg L^{-1}); COD, chemical oxygen demand (mg L^{-1}); Cond, conductivity (µS cm^{-1}); Turb, turbidity (NTU); $N\text{-}NO_3^-$, nitrate (mg L^{-1}); $N\text{-}NO_2^-$, nitrite (mg L^{-1}); $N\text{-}NH_4^+$, ammonium, (mg L^{-1}); $P\text{-}PO_4^{-3}$, phosphate (mg L^{-1}). *Piph*, picophytoplankton; *Naph*, nanophytoplankton; *Miph*, microphytoplankton; *Mizo*, microzooplankton; *Mezo*, mesozooplankton..

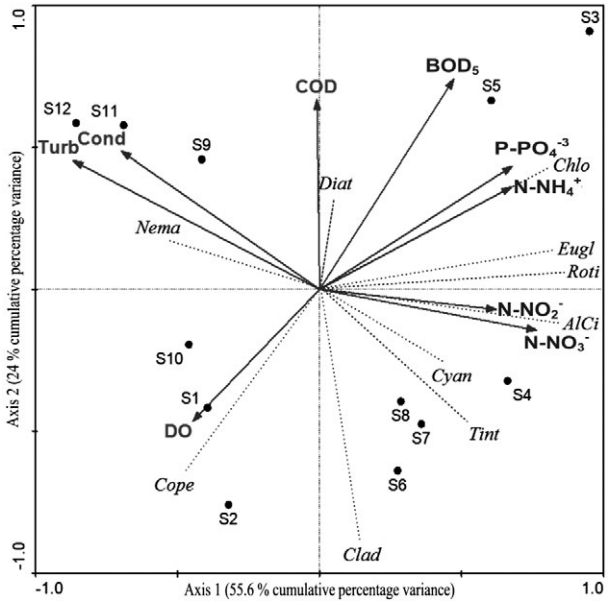

Fig. 6. Redundancy analysis (RDA) triplot of biomass of major taxonomic groups of plankton, study sites (S), and physicochemical variables. *Diat*, diatoms; *Eugl*, euglenophytes; *Chlo*, chlorophytes; *Cyan*, cyanobacteria; *Roti*, rotifers; *AlCi*, aloricate ciliates; *Cope*, copepods; *Clad*, cladocerans; *Tint*, tintinnids; *Nema*, nematodes. Abbreviations for the physicochemical variables are indicated in Fig. 5.

tertidal coast was about $3.5x10^3$ pg C mL^{-1}, but the highest biomass of the phytoplankton was *ca.* twofold higher at the most polluted area of the estuary. Nonetheless, our estimations are lower than those reported for the freshwater tidal zone ($56x10^3$ pg C mL^{-1}) as well as the mixohaline zone ($231x10^3$ pg C mL^{-1}) of the Río de la Plata estuary (Gómez *et al.*, 2004). The nanophytoplankton biomass, mostly comprised of small diatoms and cyanobacteria, represented a large proportion (84%) of the total phytoplankton biomass. A similar proportion had also been reported for other regions of the Río de la Plata estuary (Gómez *et al.*, 2004) and other communities of estuarine phytoplankton (Detmer and Bathmann, 1997; Tarran *et al.*, 2001). The biomass of picophytoplankton and nanophytoplankton (mainly consisting of chlorophytes chlorococcales, cyanobacteria, and small diatoms, respectively) was positively associated with the most polluted area characterized by high concentrations of $P\text{-}PO_4^{-3}$ and $N\text{-}NH_4^+$ and the greatest bacterial density and biomass. In agreement with Jacquet *et al.* (1998), we adopt the idea that phosphorus availability regulates the biomass and density of the smaller-sized plankton groups such as the bacteria, the pico-, and nanophytoplankton.

The highest microphytoplankton biomass, mainly composed of large diatoms, was positively correlated with study sites located within the outer part of the study area with higher levels of conductivity and turbidity. This distribution pattern was also observed by Carreto *et al.* (2003) and Calliari *et al.* (2005, 2009) in the outer zone of the Río de la Plata estuary, where lower levels of nutrients were recorded along with the highest turbidity and conductivity levels. Large cells that have a high growth-to-size relationship are generally disfavored by stratification because they need turbulence to keep them suspended in the euphotic zone (Capriulo *et al.*, 2002). The high biomass of microphytoplankton observed in areas with lower concentrations of nutrients and high turbidity may be attributable to a higher response to the fluctuating light in turbulent zones than smaller cells are capable of (Kiørboe, 1993). In the outer area of the Río de la Plata, the high speed of the tidal current and wind cause turbulence inducing a resuspension of sediments and creating a zone of maximum turbidity and salinity along with a stratification influencing flocculation and the sedimentation speed of particulate matter (Simionato *et al.*, 2011). We thus believe that the prevalence of large cells of diatoms observed in the outer sites would have contributed to an increase in the total phytoplankton biomass despite the low phytoplankton density registered in that area.

We observed that the biomass of large-sized diatoms, mainly constituted by the genera *Skeletonema*, *Actinocyclus*, *Cyclotella*, and *Aulacoseira*, represented some 81% of total phytoplankton biomass, and the biomass of this group was also greater at the coastal sites close to the mouth of the Río de la Plata estuary. This diatom assemblage had also been recorded along the fluvial-mixohaline axis of the same study system (Gómez and Bauer, 1998; Gómez *et al.*, 2004) and in the outer area of other estuaries worldwide (Mallin and Paerl, 1994; Muylaert and Sabbe, 1999; Domingues *et al.*, 2005; Guinder *et al.*, 2010). The success of these genera in competition with other taxa might be related to those diatoms' adaptive response to stressful environments. Indeed, diatoms have a higher efficiency in capturing the light within turbid habitats, a higher tolerance to a wide range of salinity levels, and a chain structure that favors resuspension through a turbulent mixing of the water column, thus delaying sedimentation to the estuary bottom (Kiørboe, 1993).

The Chlorophyta was the second most prevalent group contributing to the total biomass of the phytoplankton and were registered in a single peak at Site 3, the most eutrophic and polluted study site. We found that *Dictyosphaerium* was the more frequent genus within this taxonomic group. Consistent with our finding, this same genus had furthermore been reported to be one of the most frequent genera present in the inner section of the coastal fringe of the Río de la Plata estuary (Gómez and Bauer, 1998). We believe that the prevalence of this group, and that representation occurring in a highly environmentally disturbed area, could be related to that genus' high growth rate, nutrient uptake, and tolerance to low salinities (Paerl *et al.*, 2003).

The Cyanophyta are generally known to thrive in environments rich in nutrients and of low salinities (Paerl *et al.*, 2003; Sidik *et al.*, 2008). Nevertheless, the biomass estimated in this study represented only 1.7% of the total phytoplankton value. We observed that the most frequent genera within this group were *Oscillatoria* and *Merismopedia*. According to our estimation, these genera, as well as some potentially toxic individual species like *Microcystis aeruginosa* and *Planktothrix agardhii*, have been previously recorded in the Río de la Plata (Gómez and Bauer, 1998; Gómez *et al.*, 2004) and in other estuaries (Muylaert and Sabbe, 1999; Domingues *et al.*, 2005). Like the Chlorophytes, the cyanobacteria tolerate low salinity levels and high nutrient uptake, conditions that allow them to inhabit polluted environments. Thus, we believe that these biologic features would explain why the biomass was higher in polluted and environmentally eutrophic sites characterized by less turbulent waters than in the outer area. Although the biomass of cyanobacteria was low in the phytoplankton, the concentration of cyanotoxins produced by *M. aeruginosa* and *P. agardhii* could significantly increase with algal blooms once long residence times are establish in late spring and summer (Andrinolo *et al.*, 2007). Moreover, the mere presence of these potentially toxic species is indicative of poor water quality.

The composition, biomass, and size structure of zoo-

plankton are strongly influenced by eutrophication (Pinto-Coelho *et al.*, 2005). In this study, the biomass of microzooplankton represented about 45% of the total biomass of zooplankton. The microzooplankton in the Río de la Plata is mainly modulated by food resources; which items, in turn, are subjected to environmental conditions in each area of the estuary (Kogan, 2005). We found that the highest biomass was associated to the most polluted area in the intertidal coast of the estuary ($6.9x10^3$ pg C mL^{-1}) and coincided with high bacterial and picophytoplankton biomasses. Nevertheless, the biomass estimated in this study was five-times lower than that reported by Kogan (2005) for the fluvial-mixohaline axis ($37.8x10^3$ pg C mL^{-1}). We believe that the condition of significantly low-dissolved oxygen registered at highly eutrophic and polluted study sites might have negatively affected the abundance of microzooplankton so as to yield a lower biomass than that reported by Kogan (2005).

Organisms belonging to microzooplankton like ciliates and rotifers are capable of feeding on picophytoplankton-sized particles (Froneman, 2001). These phytoplanktonic groups along with detritus constitute the main rotifer food resource (Arndt, 1991). The redundancy analysis showed that the biomass of rotifers was related to the highly eutrophic and polluted sites and to high biomasses of chlorophytes and euglenophytes. This result is in agreement with the distribution pattern observed by Kogan (2005) for the fluvial-mixohaline axis in the Río de la Plata estuary. We also recorded microzooplanktonic species, such as *Codonella cratera*, *Vorticella* spp., and *Keratella tropica,* in sites where the nutrient concentrations and phytoplankton biomasses were high. In addition, aloricate ciliates are also capable of consuming smaller phytoplankton, including cyanobacteria, picoplanktonic chlorophytes, and bacteria (Callieri *et al.*, 2002). We likewise observed that the biomass of the aloricate ciliates was associated with higher biomasses of cyanobacteria and chlorophytes and in close relationship with study sites having higher concentrations of $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$. Furthermore, tintinnids have also been reported to have a higher density and biomass at sites with lower salinity and higher nutrient concentrations (Pierce and Turner, 1994; Kogan, 2005). We accordingly observed the highest biomass of tintinnids at sites with higher concentrations of nutrients.

The mesozooplankton biomass represented 55% of the total zooplankton value and was mainly constituted by planktonic copepods that were associated with sites located at innermost part of the estuary, though we did find a peak of biomass at Site 10. In general, planktonic copepods prefer to feed on cells larger than 10 µm (Bautista and Harris, 1992). For instance, diatoms such as *Thalassiosira* spp., *Cyclotella meneghiniana*, and *Skeletonema costatum* are the most frequently selected food source for copepods and other zooplankton groups (Mallin and Paerl, 1994). We found that central diatoms dominated at sites located in the middle and outer area of the intertidal coast of the estuary. This trophic interaction might explain why we observed a peak of copepods' biomass at Site 10. According to this observation, we would have expected a significant association between copepods and diatoms, but the redundancy analysis showed no significant correlation between them. For instance, the diatom biomass was the lowest at the innermost part of the estuary, where we found the copepod biomass to be two-times higher in that area than in the outer region.

CONCLUSIONS

This work constitutes the first ecological study analyzing the spatial distribution of density and biomass of the plankton community along the freshwater intertidal coast of the Río de la Plata estuary. The evidence presented in this study would indicate that the eutrophication and organic pollution resulting from anthropic inputs increases the density and biomass of planktonic organisms such as the bacteria, the small-sized phytoplankton, and the microzooplankton species. Thus, the differences found in the taxonomic composition and size structure of the planktonic-community assemblage between the most polluted and less polluted sites highlights the significant impact of human activities in modifying the functioning of this coastal ecosystem.

Nevertheless, in order to understand completely the causality of the spatial variation documented in the present study, experimental manipulations, both *in situ* and *in vitro*, of the multitude of conditions and influences that could conceivably affect the size fraction and major taxonomic groups of intertidal plankton would be needed. We consider, however, that our findings provide new information on the utility of plankton as an indicator of water quality in estuaries and would constitute an excellent baseline for further ecological and water-quality research.

ACKNOWLEDGMENTS

We thank the anonymous reviewers for their critical review and helpful suggestions of the manuscript. We want also to express our gratitude to Drs. Mónica Hoffmeyer and Valeria Guinder for their detailed comments and corrections. Dr. Donald F. Haggerty, a retired career investigator and native English speaker, edited the final version of the manuscript. Financial support for this study was provided by the grant PICT 32077 to Dr. Nora Gómez, head of the laboratory where this work was done. MDG is a doctoral fellow in CONICET; NB is a postdoc fellow in CONICET.

REFERENCES

Alonso C, Gómez-Pereira P, Ramette A, Ortega L, Fuchs BM, Amann R, 2010. Multilevel analysis of the bacterial diver-

sity along the environmental gradient Rio de la Plata-South Atlantic Ocean. Aquat. Microb. Ecol. 61:57-72.

APHA, 1998. Standard methods for examination of water and wastewater. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington: 1220 pp.

Andrinolo D, Pereira P, Giannuzzi L, Aura C, Massera S, Caneo M, Caixach J, Barco M, Echenique R, 2007. Ocurrence of *Microcystis aeruginosa* and microcystins in the Río de la Plata (Argentina). Acta Toxicol. Arg. 15:13-18.

Arndt H, 1991. Population dynamics and production of estuarine planktonic rotifers in the southern Baltic: *Brachionus quadridentatus* (Hermann, 1783). Acta Ichthyol. Piscatoria 21:7-15.

Bautista B, Harris RP, 1992. Copepod gut contents, ingestion rates and grazing impact on phytoplankton in relation to size structure of zooplankton and phytoplankton during a spring bloom. Mar. Ecol. Prog. Ser. 82:41-50.

Beaugrand G, 2005. Monitoring pelagic ecosystems using plankton indicators. ICES J. Mar. Sci. 62:333-338.

Beers JR, Stewart GL, 1970. Numerical abundance and estimated biomass of microzooplankton. In: J.D.H. Strickland (ed.) The ecology of the plankton off La Jolla, California, in the period April through September 1967. Bull. Scripps Inst. Oceanogr. 17:67-87.

Borja A, Basset A, Bricker S, Dauvin JC, Elliot M, Harrison T, Marques JC, Weisberg SB, West R, 2012. Classifying ecological quality and integrity of estuaries. Treatise on Estuarine and Coastal Science 1:125-162.

Bottrell H, Duncan A, Gliwicz Z, Grygierek E, Herzig A, Hillbricht-Ilkowska A, Kurasawa H, Larsson P, Weglenska T, 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. 24:419-456.

Bratbak G, Dundas I, 1984. Bacterial dry matter content and biomass estimations. Appl. Environ. Microb. 48:755-757.

Calliari D, Brugnoli E, Ferrari G, Vizziano D, 2009. Phytoplankton distribution and production along a wide environmental gradient in the South-West Atlantic off Uruguay. Hydrobiologia 620:47-61.

Calliari D, Gómez M, Gómez N, 2005. Biomass and composition of the phytoplankton in the Río de la Plata: large-scale distribution and relationship with environmental variables during a spring cruise. Cont. Shelf Res. 25:197-210.

Callieri C, Karjalainen SM, Passoni S, 2002. Grazing by ciliates and heterotrophic nanoflagellates on picocyanobacteria in Lago Maggiore, Italy. J. Plankton Res. 24:785-796.

Capriulo GM, Smith G, Troy R, Wikfors GH, Pellet J, Yarish C, 2002. The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. Hydrobiologia 475/476:263-333.

Carreto JI, Montoya NG, Benavides HR, Guerrero R, Carignan MO, 2003. Characterization of spring phytoplankton communities in the Río de La Plata maritime front using pigment signatures and cell microscopy. Mar. Biol. 143:1013-1027.

De Jonge VN, Elliot M, Orive E, 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. Hydrobiologia 475/476:1-19.

Detmer AE, Bathmann UV, 1997. Distribution patterns of autotrophic pico- and nanoplankton and their relative contribution to algal biomass during spring in the Atlantic sector of the Southern Ocean. Deep-Sea Res. Pt. II 44:299-320.

Domingues RB, Barbosa A, Galvão H, 2005. Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). Estuar. Coast. Shelf. S. 64: 249-260.

Dumont H, Van De Velde I, Dumont S, 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19:75-97.

Dutto MS, López Abbate MC, Biancalana F, Berasategui AA, Hoffmeyer MS, 2012. The impact of sewage on environmental quality and the mesozooplankton community in a highly eutrophic estuary in Argentina. ICES J. Mar. Sci. 69:399-409.

Feller RJ, Warwick RM, 1988. Energetics, p. 181-196. In: R.P. Higgins and G. Thiel (eds.), Introduction to the study of meiofauna. Smithsonian Institution Press.

Framiñán MB, Brown OB, 1996. Study of the Río de la Plata turbidity front, Part I: spatial and temporal distribution. Cont. Shelf Res. 16:1259-1289.

Froneman PW, 2001. Seasonal changes in zooplankton biomass and grazing in a temperate estuary, South Africa. Estuar. Coast. Shelf S. 52:543-553.

Fuhrman JA, Azam F, 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation of field results. Mar. Biol. 66:109-120.

Gómez N, Bauer DE, 1998. Phytoplankton from the ''Southern Coastal Fringe'' of the Río de la Plata (Buenos Aires, Argentina). Hydrobiologia 380:1-8.

Gómez N, Bauer DE, Licursi M, Hualde PR, 2002. Planktonic and periphytic coastal algae of the Río de La Plata, Argentina. Int. Ver. Theor. Angew. 28:250-253.

Gómez N, Hualde PR, Licursi M, Bauer DE, 2004. Spring phytoplankton of Río de la Plata: a temperate estuary of South America. Estuar. Coast. Shelf. S. 61:301-309.

González-Ortegón E, Pascual E, Cuesta JA, Drake P, 2006. Field distribution and osmoregulatory capacity of shrimps in a temperate European estuary (SW Spain). Estuar. Coast. Shelf. S. 67: 293-302.

Guerrero MA, Acha ME, Framiñan MB, Lasta C, 1997. Physical oceanography of the Río de la Plata Estuary. Cont. Shelf Res. 17:727-742.

Guinder VA, Popovich CA, Molinero JC, Perillo GME, 2010. Long-term changes in phytoplankton phenology and community structure in the Bahía Blanca Estuary, Argentina. Mar. Biol. 157:2703-2716.

Hagstrøm A, Larsson U, Horstedt P, Norwalk S, 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl. Environ. Microb. 37:805-812.

Heinbokel JF, Coats DW, Henderson KW, Tyler MA, 1988. Reproduction rates and secondary production of three species of the rotifer genus *Synchaeta* in the estuarine Potomac River. J. Plankton Res. 10:659-674.

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T, 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35:403-424.

Jacquet S, Lennon JF, Marie D, Vaulot D, 1998. Picoplankton population dynamics in coastal waters of the northwestern Mediterranean Sea. Limnol. Oceanogr. 43:1916-1931.

Jaime P, Menéndez AN, Natale OE, 2001. [Balance y dinámica de nutrientes principales en el Río de la Plata interior].[Report in Spanish]. Instituto Nacional del Agua, Argentina. Available from: http://www.ina.gov.ar/pdf/LH-it_rdplata_balance_ sep01.pdf

Kiørboe T, 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Adv. Mar. Biol. 29:2-61.

Kogan M, 2005. [Estudio de la composición específica, abundancia y distribución especial del microzooplancton (Protozoos y micrometazoos) en el estuario del Río de La Plata (Argentina/Uruguay)].[Thesis in Spanish]. Universidad Nacional de Buenos Aires.

Kromkamp J, Peene J, 2005. Changes in phytoplankton biomass and primary production between 1991 and 2001 in the Westerschelde estuary (Belgium/The Netherlands). Hydrobiologia 540:117-126.

Kurucz A, Masello A, Méndez S, Cranston P, Wells PG, 1998. [Calidad ambiental del Río de La Plata, p. 71-86]. In: P.G. Wells and G.R. Daborn (eds.), El Río de La Plata, una revisión ambiental. Un informe de Antecedentes del Proyecto EcoPlata].[Book in Spanish]. Dalhousie University, Halifax.

Lepš J, Šmilauer P, 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press.

Lund JWG, Kipling C, Le Cren ED, 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143-170.

Mackereth FJH, Heron J, Talling JF, 1978. Water analysis: some revised methods for limnologists. Freshwater Biological Association: 120 pp.

Mallin MA, Paerl HW, 1994. Planktonic trophic transfer in an estuary: seasonal, diel, and community structure effects. Ecology 75:2168-2184.

Margalef R, 1955. [Los organismos indicadores en la limnología. Biología de las aguas continentales].[Book in Spanish]. Ministerio de Agricultura, Instituto Forestal de Investigaciones y Experiencias: 300 pp.

Margalef R, 1983. [Limnología].[Book in Spanish]. Omega, Barcelona: 1010 pp.

Marques SC, Azeiteiro UM, Leandro SM, Queiroga H, Primo AL, Martinho F, Viegas I, Pardal MÂ, 2008. Predicting zooplankton response to environmental changes in a temperate estuarine ecosystem. Mar. Biol. 155:531-541.

Mc Cauley E, 1984. The estimation of the abundance and biomass of zooplankton in samples, p. 228-265. In: J. Downing and F. Rigler (eds.), A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications.

Menden-Deuer S, Lessard EJ, 2000. Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. Limnol. Oceanogr. 45:569-579.

Mianzan H, Lasta C, Acha E, Guerrero R, Macchi G, Bremec C, 2001. The Río de la Plata Estuary, Argentina-Uruguay, p. 185-204. In: U. Seeliger and B. Kjerfve (eds.) Coastal marine ecosystems of Latin America. Ecological studies. Springer.

Muylaert K, Sabbe K, 1999. Spring phytoplankton assemblages in and around the maximum turbidity zone of the estuaries of the Elbe (Germany), the Schelde (Belgium/The Netherlands) and the Gironde (France). J. Marine Syst. 22:133-149.

Norland S, 1993. The relationship between biomass and volume of bacteria, p. 303-307. In: P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole, (eds.) Handbook of methods in aquatic microbial ecology. Lewis Publishers.

Paerl HW, Valdes LM, Pinckney JL, Piehler MF, Dyble J, Moisander PH, 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. BioScience 553:953-964.

Paerl HW, Rossignol KL, Hall SN, Peierls BL, Wetz MS, 2010. Phytoplankton community indicators of short-and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuar. Coast. 33:485-497.

Pierce RW, Turner JT, 1994. Plankton studies in Buzzards Bay, Massachusetts, USA. IV. Tintinnids, 1987 to 1988. Mar. Ecol.-Prog. Ser. 112:235-240.

Pinto-Coelho RM, Bezerra-Neto JF, Morais-Jr CA, 2005. Effects of eutrophication on size and biomass of zooplankton in a tropical reservoir. Braz. J. Biol. 65:325-338.

Porter KG, Feig YS, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25:943-948.

Putt M, Stoecker DK, 1989. An experimentally determined carbon: volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34:1097-1103.

Riemann F, Ernst W, Ernst R, 1990. Acetate uptake from ambient water by the free-living marine nematode *Adoncholaimus thalassophygas*. Mar. Biol. 104:453-457.

Roggiero MF, 1988. [Fitoplancton del Río de la Plata, I].[Article in Spanish]. Lilloa 37:137-152.

Ruttner-Kolisko A, 1977. Suggestions for biomass calculations of plankton rotifers. Archr. Hydrobiol. 8:71-76.

Sidik MJ, Rashed-Un-Nabi Md, Azharul Hoque Md, 2008. Distribution of phytoplankton community in relation to environmental parameters in cage culture area of Sepanggar Bay, Sabah, Malaysia. Estuar. Coast. Shelf. S. 80:251-260.

Simionato CG, Moreira D, Piedra-Cueva I, Fossati M, Re M, Sabarots Gerbec M, Menendez AN, Cayocca F, 2011. [Proyecto FREPLATA-FFEM Modelado numérico y mediciones in-situ y remotas de las transferencias de sedimentos finos a través del Río de la Plata. Parte B: Simulaciones numéricas].[Article in Spanish]. Revista Frente Marítimo 22:265-304.

Tarran GA, Zubkov MV, Sleigh MA, Burkill PH, Yallop M, 2001. Microbial community structure and standing stocks in the NE Atlantic in June and July of 1996. Deep-Sea Res. Pt II 448:963-985.

Ter Braak CJF, Verdonschot PFM, 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. 57:255-289.

Verity PG, Langdon C, 1984. Relationship between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J. Plankton Res. 6:859-868.

Verlecar XN, Desai SR, Sarkar A, Dalal SG, 2006. Biological indicators in relation to coastal pollution along Karnataka coast, India. Water Res. 40:3304-3312.

Wieser W, 1960. Benthic studies in Buzzards Bay. II. The meiofauna. Limnol. Oceanogr. 5:121-137.

Wilson JG, 1994. The role of bioindicators in estuarine management. Estuaries 17:94-101.